Optogenetic Modulation of Neural Circuits That Underlie Reward-Seeking

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Introduction
The manifestation of complex neuropsychiatric disorders, such as drug and alcohol addiction, is thought to result from progressive maladaptive alterations in neural circuit function. Repeated drug exposure has been shown to alter distributed network of neural circuit elements. However, a more precise understanding of addiction has been hampered by an inability to control and, consequently, to identify specific circuit components that underlie addictive behaviors. The development of optogenetic strategies for selectively modulating the activity of genetically defined neuronal populations has provided a means for determining the relationship between circuit function and behavior with a level of precision that was previously unobtainable.

In this chapter, we briefly review the main optogenetic studies that have contributed to elucidating neural circuit connectivity within the ventral tegmental area (VTA) and the nucleus accumbens (NAc)—two brain nuclei that are essential for the manifestation of addiction-related behaviors. Additional targeted manipulation of genetically defined neural populations in these brain regions, as well as afferent and efferent structures, promises to delineate the cellular mechanisms and circuit components required for an organism to transition from natural goal-directed behavior to compulsive reward-seeking, despite its negative consequences.

Optogenetics for Studying Complex Neural Systems
The organizational complexity of the brain is both a source of its computational power and the main obstacle to our understanding neural systems and the development of therapeutics for neuropsychiatric diseases, such as addiction. Although the basic neuroanatomical substrates required for reward-related behavior have been identified, the specific function of genetically defined neurons and neural circuits remains unclear. To define the functional connectivity between neurons and their role in modulating complex behaviors, such as reward-seeking, requires the ability to perturb specific neural circuits on physiologically relevant timescales. Given the complexity and high degree of interconnectivity within neural tissue, manipulating the activity of a single, genetically defined neuronal cell type in heterogeneous tissue has been a major obstacle. The development of optogenetic strategies, however, has allowed for selective activation and inhibition of genetically defined circuit elements with millisecond resolution. This ability has circumvented many of the technical limitations of traditional techniques used in systems and behavioral neuroscience research. A new level of mechanistic insight into the neural underpinnings of motivated behaviors is now possible.

Two highly interconnected brain regions play critical roles in mediating reward-seeking behaviors, including those related to addiction: the VTA and the NAc. These brain regions comprise multiple, genetically distinct cell groups that integrate and convey reward-related information. The following sections briefly introduce the use of optogenetics to study neural circuit function. Next, they provide an overview of the synaptic connectivity within the VTA and NAc and highlight how optogenetic studies have further delineated neural circuit function within these regions.

Tools and Strategies for Optogenetic Manipulation of Neural Circuits That Underlie Reward Processing
Several optogenetic actuators are now available for both excitation and inhibition of neural circuits for use both in vitro and in vivo (Yizhar et al., 2001). To date, the most commonly used light-gated proteins for activating neural tissue are engineered mutants of channelrhodopsin-2 (ChR2) (Boyden et al., 2005). ChR2 mutants are typically maximally activated by 450–500 nm light, which allows for large inward flux of Na+ and Ca2+ at resting membrane potentials. Brief pulses of light (typically 1–5 ms) result in reliable and repeatable action potential generation in a variety of neuronal subtypes over a large range of firing frequencies (Tsai et al., 2009; Stuber et al., 2011). Expression of ChR2 in neuronal fibers can also be used to selectively activate pathway-specific neurotransmitter release in brain slices (Petreanu et al., 2007; Stuber et al., 2010, 2011) or in behaving animals (Stuber et al., 2011; Tye et al., 2011) to study the effects of afferent-specific synaptic transmission. For optogenetic inhibition studies, modified variants of both halorhodopsin (Han et al., 2007; Zhang et al., 2007; Gradinaru et al., 2010) and archaerhodopsin (Chow et al., 2010; Han et al., 2011) have been shown to reliably silence neural activity both in vitro and in vivo. Transgenes coding for gated proteins to modulate neural activity are typically introduced into neural tissue via transgenic animals that express these proteins under cell-type-specific neuronal promoters (Wang et al., 2007; Zhao et al., 2011), or, more commonly, by recombinant viral vectors that can be stereotactically delivered to discrete brain nuclei. For delivering light to neural tissue in vivo, optical fibers, coupled with high-powered light sources such as lasers or LEDs, are used (Cardin et
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30

al., 2010; Zhang et al., 2010; Sparta et al., 2011). This provides a way to restrict light delivery and thus optical modulation to specific brain structures. Combined with genetic targeting approaches to selectively express these light-activated proteins in genetically defined neurons (Tsai et al., 2009), use of these tools allows for selective modulation of neural circuits that underlie reward-related behaviors.

Neuronal Populations in the VTA and Their Role in Mediating Reward-Seeking Behavior

The VTA is a heterogeneous brain structure containing neuronal populations that are essential to the expression of motivated behaviors related to addiction (Wise, 2004; Fields et al., 2007). While the VTA is often treated as a distinct neural structure, few anatomical markers distinguish it from neighboring structures such as the substantia nigra pars compacta (SNc). Immunohistochemical and tracing studies have suggested that the SNc comprises a relatively homogenous population of neurons, the majority of which are dopaminergic (DAergic) (90%) and project to the dorsal striatum (Swanson, 1982; Margolis et al., 2006). The VTA, on the other hand, contains a mixture of DAergic (~65%), GABAergic (~30%), and glutamatergic neurons (~5%) (Dobi et al., 2010) that project throughout the forebrain to structures including the amygdala, prefrontal cortex, and NAc (Swanson, 1982). Importantly, VTA neurons that project to the NAc are a heterogeneous population of both DAergic and GABAergic neurons (Van Bockstaele and Pickel, 1995; Carr and Sesack, 2000a; Margolis et al., 2006). Within the VTA, GABAergic neurons also are thought to form inhibitory contacts onto at least some DAergic projection neurons (Johnson and North, 1992). Thus, VTA GABAergic neurons, as well as GABAergic input from the posterior segment of the VTA, also known as the rostromedial tegmental nucleus (RMTg) (Jhou et al., 2009a,b), may play an important role in regulating DAergic neuron function. These diverse neuronal populations in the VTA are likely components of distinct neural circuits incorporating neurons from their afferent and efferent structures (discussed in detail below), which may act to mediate specific aspects of motivated behavioral processing (Lammel et al., 2011).

The pioneering optogenetic studies in this field introduced the blue light–sensitive cation channel ChR2 exclusively into VTA DAergic neurons using viral delivery methods to examine the role of these specific VTA neurons in reward-seeking behavior (Tsai et al., 2009; Brown et al., 2010; Stuber et al., 2010; Aamantidis et al., 2011). While the actions of DA were known to play an important role in reward-related behaviors, it was not possible to study how the selective activation of DAergic neurons alone could modulate reward-related behavior. Noncontingent, high-frequency, optical activation of VTA DAergic neurons led to the formation of a conditioned place preference (CPP) to the associated environment (Tsai et al., 2009). Significantly, DAergic stimulation frequencies that led to the development of a CPP also resulted in transient surges of DA release in the NAc, suggesting that only stimulation frequencies that lead to detectable changes in DA release can induce associative learning. In addition, it has been demonstrated that direct optical activation of these neurons can reinforce operant behavioral responding (Witten et al., 2011) and facilitate the development of positive reinforcement (Adamantidis et al., 2011). Taken together, these studies demonstrate that direct activation of VTA DAergic neurons alone can promote behavioral conditioning and reinforce behavioral response in the absence of any additional reward.

Optogenetic strategies have also been employed in brain-slice experiments to examine the possibility of neurotransmitter corelease. Neurons in the medial VTA coexpress tyrosine hyrolylase and the vesicular glutamate transporter-2 (VGLuT2), indicating that they are capable of both synthesizing DA and packaging glutamate into synaptic vesicles (Hnasko et al., 2010). Prior to optogenetic manipulations, however, it was not possible to selectively stimulate DAergic fibers originating from VTA neurons to determine whether they corelease dopamine and other small-molecule neurotransmitters. Optogenetic stimulation of DAergic terminals in the NAc led to detectable glutamate-mediated excitatory postsynaptic currents (Stuber et al., 2010; Tecuapetla et al., 2010) that were not present in mice that lack VGLuT2 in DAergic neurons (Stuber et al., 2010). Further, glutamate release was not detected in dorsal striatal regions despite optogenetic stimulation of DAergic fibers producing detectable DA release (Stuber et al., 2010). These studies suggest that midbrain DAergic neurons that project to the ventral but not dorsal striatum can corelease glutamate as a neurotransmitter. Interestingly, a recent electron microscopy study demonstrated that axonal fibers within different striatal subregions do not coexpress TH and VGLuT1, VGLuT2, or VGLuT3 (Moss et al., 2011). These results suggest there may be major species-specific differences in the neurotransmitter content of DAergic neurons (the
Optogenetic studies were performed in mice, whereas the electron microscopy study was performed in rats). However, it is worth noting that cultured DAergic neurons from rats also corelease glutamate (Sulzer et al., 1998; Joyce and Rayport, 2000) and that electrical stimulation of the VTA results in glutamate-mediated EPSPs in the prefrontal cortex of rats (Lavin et al., 2005). These findings suggest that DAergic neurons in species other than mice also corelease glutamate. It is possible that different axonal fibers that originate from a single DAergic neuron in the VTA may release DA or glutamate, but not both. In addition, transcriptional suppression of TH may occur in VTA neurons that coexpress VGluT2. Although further studies are required, these ideas could account for the discrepancies between the optogenetic studies demonstrating DA/glutamate corelease and the electron microscopy data showing that axonal fibers in the striatum do not coexpress TH and VGluT isoforms.

Excitatory afferent projections to the VTA

Direct optogenetic stimulation of VTA DAergic neurons has demonstrated unequivocally that activation of these neurons is sufficient to modulate reward-related behaviors. Thus, an important line of research that remains largely unexplored is determining how specific VTA afferents modulate the activity of both DAergic and non-DAergic neurons in the VTA. Both excitatory and inhibitory afferents from a number of nuclei (Fig. 1) innervate postsynaptic neurons within the VTA. The heterogeneity of these inputs is such that electrical stimulation cannot be used to activate specific presynaptic fibers. Therefore, future studies will have to rely on afferent-specific optogenetic stimulation to study pathway-specific synaptic function in the VTA. Optogenetic stimulation of presynaptic fibers in other brain regions has already uncovered novel functions of afferent-specific inputs (Petreanu et al., 2007; Stuber et al., 2011; Tye et al., 2011). Exposure to many drugs and natural rewards can alter excitatory synaptic function onto midbrain DAergic neurons (Ungless et al., 2001; Saal et al., 2003; Borgland et al., 2004; Bellone and Luscher, 2006; Mameli et al., 2007; Chen et al., 2008; Stuber et al., 2008; Lammel et al., 2011). Therefore, further identifying the exact synaptic connectivity to different populations of VTA neurons should provide a framework for better understanding the synaptic mechanism by which natural rewards and drug abuse alter synaptic function within the VTA.

Lateral hypothalamus

The lateral hypothalamus (LH) is thought to send the largest subcortical glutamatergic projection to the VTA/SNc (Geisler et al., 2007). Electrical stimulation of the LH increases the firing rates predominately of VTA/SNc neurons that display long-duration action potential waveforms (Maeda and Mogenson, 1981). In contrast, neurons that show short-duration waveforms are generally suppressed by LH stimulation (Maeda and Mogenson, 1981). While most of the fibers originating from the LH and projecting to the VTA are glutamatergic (Geisler et al., 2007) and may corelease the neuropeptide orexin (Aston-Jones et al., 2010), whether there is a GABAergic input from the LH to the VTA is unknown. VTA-projecting LH neurons show increased activity as indexed by c-fos following cocaine or morphine CPP (Harris et al., 2005, 2007), and VTA neurons show increases in c-fos expression following LH stimulation (Arvanitogiannis et al., 1997). In addition, LH self-stimulation leads to large increases in NAc DA release (Hernandez and Hoebel, 1988), further demonstrating an important role of this pathway in the activation of brain reward circuits as well as reinforcing behavioral responding. Taken together, these studies show that the LH is an

Figure 1. Circuitry of the ventral tegmental area. VTA DAergic neurons project to forebrain targets such as the basolateral amygdala (BLA), mPFC, and NAc. These neurons received excitatory synaptic inputs from the LH, mPFC, and PPTg/LDT. Inhibitory inputs to the VTA neurons also arise from extended amygdala output structures. VTA GABAergic neurons target neighboring DAergic neurons as well as projecting to the mPFC and NAc. These neurons are thought to receive excitatory inputs from the LH and inhibitory inputs from the NAc. RMTg, rostromedial tegmental nucleus. Reprinted from Stuber GD et al. (2012) Biological Psychiatry. 71(12):1061–1067, their Figure 1.
important source of excitatory drive to the VTA. It is hoped that future studies that involve afferent-specific optogenetic stimulation of LH afferents to the VTA will illuminate the synaptic connectivity between these regions as well as their role in reward-related behaviors.

**Medial prefrontal cortex**

Another major source of glutamatergic input to the VTA comes from a long-range projection from the medial prefrontal cortex (mPFC), which is thought to target both DAergic and non-DAergic neurons (Sesack and Pickel, 1992; Carr and Sesack, 2000b; Geisler et al., 2007). Stimulation of the mPFC performs a variety of functions: It leads to an increase in extracellular glutamate in the VTA (You et al., 2007), activates DAergic and non-DAergic neurons (Gariano and Groves, 1988; Tong et al., 1996; Moorman and Aston-Jones, 2010), and elevates DA release in the forebrain (Karreman and Moghaddam, 1996; You et al., 1998). Interestingly, an electron microscopy study by Carr and Sesack (2000b) showed that mPFC afferents to the VTA form synapses onto mPFC-projecting, but not NAc-projecting, DAergic neurons. mPFC afferents also formed synapses onto VTA GABAergic neurons that project to the NAc but not those that project to the mPFC. Afferent-specific optogenetic stimulation with postsynaptic recordings from DAergic and non-DAergic neurons in the VTA is needed to corroborate these findings.

**Lateral habenula**

The lateral habenula (LHb) is another major excitatory input to the VTA and an important modulator of DAergic neuronal activity (Christoph et al., 1986; Matsumoto and Hikosaka, 2007; Bromberg-Martin et al., 2010). Glutamatergic fibers originating in the LHb project directly to the VTA (Herkenham and Nauta, 1979; Geisler et al., 2007) and are thought to form synapses on both DAergic and GABAergic neurons there (Omelchenko et al., 2009). Interestingly, electrophysiological recordings from LHb neurons in behaving monkeys have demonstrated that these neurons are predominantly inhibited during reward expectation, while DAergic neurons in the midbrain show excitatory responses (Matsumoto and Hikosaka, 2007, 2009; Bromberg-Martin et al., 2010). In addition, LHb neurons are excited by aversive stimuli (Matsumoto and Hikosaka, 2007), and stimulation of the LHb leads to reduced DA release in the NAc (Lisoprawski et al., 1980). These studies suggest that LHb neurons send a direct glutamatergic projection to predominantly GABAergic neurons in the VTA/SNc that can inhibit DAergic neuronal activity.

**Pedunculopontine tegmental nucleus**

The VTA also receives a mixed glutamatergic/cholinergic projection from the pedunculopontine tegmental nucleus (PPTg) and the laterodorsal tegmental nucleus (LDT) (Hallanger and Wainer, 1988; Futami et al., 1995; Omelchenko and Sesack, 2005). Consistent with this, electrical stimulation of PPTg/LDT increases the activity of VTA neurons and increases DA release in the NAc (Floresco et al., 2003). While the PPTg/LDT plays an important role in driving drug-seeking behavior (Schmidt et al., 2009), how the synaptic connectivity between the PPTg/LDT and the VTA controls reward-related behaviors remains unknown.

**Inhibitory Afferents to the VTA/SNc**

Within the VTA, afferent-specific optogenetic stimulation experiments examining the synaptic connectivity between neurons have demonstrated that distal GABAergic neurons originating in the NAc form functional inhibitory synaptic contacts onto non-DAergic neurons in the VTA (Xia et al., 2011). Furthermore, some non-DAergic neurons that receive inhibitory inputs from the NAc were shown to project back to the NAc (Xia et al., 2011). This study elegantly demonstrated the precise functional inhibitory connectivity between the NAc to VTA GABAergic neurons. Other neurons throughout the extended amygdala, such as those from the central nucleus of the amygdala (CeA) and the bed nucleus of the stria terminalis (BNST), were also found to project to the VTA (Georges and Aston-Jones, 2001; Jalabert et al., 2009; Lee et al., 2011). While electrical stimulation of the BNST produces both excitatory and inhibitory responses in the VTA (Georges and Aston-Jones, 2001), we have recently demonstrated that the BNST sends a mixed glutamatergic and GABAergic projection that preferentially innervates non-DAergic neurons in the VTA (Jennings et al., 2013). Local VTA GABAergic activity is also a potent modulator of DAergic output. The specific role for these distinct pathways in regulating VTA neural activity in vivo and during behavioral tasks should be explored further.

**Conclusions**

While optogenetic manipulations of brain reward circuitry have already helped establish and refute many hypotheses that were previously untestable with traditional techniques, studies that will expand our understanding of the mechanisms of neural circuits that underlie reward-seeking behavior are yet to come. Most of the studies reviewed utilized ChR2
for optogenetic stimulation of defined neuronal populations. While these early studies have certainly provided groundbreaking findings, many of them failed to take advantage of one of the greatest benefits of optogenetic methodologies: That is, they did not make use of transient activation or inactivation of neural tissue time-locked to discrete and infrequent behaviorally relevant events. In addition, while optogenetic stimulation experiments can demonstrate that synchronous activation of neurons can modulate behaviors related to reward-seeking, they do not demonstrate the necessity of specific neural circuit elements in behavior. Loss-of-function experiments utilizing optogenetic inhibition of neural circuits have lagged behind the adoption of ChR2-mediated stimulation, partially owing to technical limitations that have been recently overcome (Xia et al., 2011). These future studies will likely play a more prominent part in determining the role of specific connections between neurons in mediating motivated behavioral responses. Finally, a plethora of in vivo electrophysiological and neurochemical studies has elegantly shown correlations between neural activity and reward-seeking behavior. As optogenetic strategies for neural circuit manipulation are refined, these techniques to monitor neural activity will certainly become fully integrated with optogenetic circuit manipulation for assessing how neuronal actuation can alter neurophysiological and neurochemical responses observed during reward-seeking behavior. Taken together, it is likely that optogenetic strategies for perturbation of neural function, used in conjunction with sophisticated behavioral paradigms to study reward-seeking, will greatly enhance our understanding of the circuitry that mediates behavior.

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References


Carr DB, Sesack SR (2000a) GABA-containing neurons in the rat ventral tegmental area project to the prefrontal cortex. Synapse 38:114–123.


